

JUN. 15. 2005 1:20PM

RECEIVED
CENTRAL FAX CENTER

NO. 9414 P. 2

In re Application of: Luis PASAMONTES and Yuri TSYGANKOV
Serial No.: 10/895,980
Filed: October 29, 2003
For: FERMENTATIVE CAROTENOID PRODUCTION

JUN 15 2005

****FOR DISCUSSION ONLY - NOT FOR ENTRY ON THE RECORD****

Claims 1-4 (cancelled).

Claim 5 (currently amended). A process for the preparation of canthaxanthin wherein the said process comprises culturing a recombinant cell containing farnesyl pyrophosphate and isopentyl pyrophosphate under culture conditions sufficient for the expression of enzymes which catalyze the conversion of the farnesyl pyrophosphate and isopentyl pyrophosphate to canthaxanthin, the said recombinant cell being a host cell transformed by an expression vector comprising a regulatory sequence and a polynucleotide containing the following DNA sequences which encode the said enzymes:

- a) a DNA sequence which encodes the geranylgeranyl pyrophosphate (GGPP) synthase of *Flavobacterium* sp. R1534 (crtE) (SEQ ID NO: 2) or a DNA sequence that hybridizes to a complementary strand of SEQ ID NO: 1 under the following conditions: hybridization in 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA), 0.5 M Na₂HPO₄, pH 7.2, at 65°C, washing twice for 5 minutes each in 2X SSC, 1% SDS, at room temperature, followed by two additional washes for 15 minutes each in 0.1% SSC, 0.1% SDS, at 65°C, wherein the hybrid DNA encodes a polypeptide having geranylgeranyl pyrophosphate (GGPP) synthase activity which is substantially homologous,
- b) a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) (SEQ ID NO: 3) or a DNA sequence that

Application No.: 10/066,007
Amendment Dated: October 13, 2004
Reply to Interviews of: October 6, 2004 and October 8, 2004

hybridizes to SEQ ID NO: 1 under the following conditions: hybridization in 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA), 0.5 M Na₂HPO₄, pH 7.2, at 65°C, washing twice for 5 minutes each in 2X SSC, 1% SDS, at room temperature, followed by two additional washes for 15 minutes each in 0.1% SSC, 0.1% SDS, at 65°C, wherein the hybrid DNA encodes a polypeptide having prephytoene synthase activity which is substantially homologous,

- c) a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI) (SEQ ID NO: 4) or a DNA sequence that hybridizes to SEQ ID NO: 1 under the following conditions: hybridization in 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA), 0.5 M Na₂HPO₄, pH 7.2, at 65°C, washing twice for 5 minutes each in 2X SSC, 1% SDS, at room temperature, followed by two additional washes for 15 minutes each in 0.1% SSC, 0.1% SDS, at 65°C, wherein the hybrid DNA encodes a polypeptide having phytoene desaturase activity which is substantially homologous,
- d) a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) (SEQ ID NO: 5) or a DNA sequence that hybridizes to SEQ ID NO: 1 under the following conditions: hybridization in 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA), 0.5 M Na₂HPO₄, pH 7.2, at 65°C, washing twice for 5 minutes each in 2X SSC, 1% SDS, at room temperature, followed by two additional washes for 15 minutes each

Application No.: 10/066,007
Amendment Dated: October 13, 2004
Reply to Interviews of: October 6, 2004 and October 8, 2004

in 0.1% SSC, 0.1% SDS, at 65°C, wherein the hybrid DNA encodes a polypeptide having lycopene cyclase activity which is substantially homologous,

- e) a DNA sequence which encodes the β -carotene β 4-oxygenase of microorganism E-396 (crtW_{E396}) (SEQ ID NO: 32) or a DNA sequence that hybridizes to a complementary strand of SEQ ID NO: 31 under the following conditions: hybridization in 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA), 0.5 M Na₂HPO₄, pH 7.2, at 65°C, washing twice for 5 minutes each in 2X SSC, 1% SDS, at room temperature, followed by two additional washes for 15 minutes each in 0.1% SSC, 0.1% SDS, at 65°C, wherein the hybrid DNA encodes a polypeptide having β -carotene β 4-oxygenase activity which is substantially homologous;

and isolating the canthaxanthin from such cells or the culture medium.

Claim 6 (currently amended). ~~A~~ The process according to of claim 5 wherein the said DNA sequences are:

- (a) the a DNA sequence which encodes the GGPP synthase of *Flavobacterium* sp. R1534 (crtE) (SEQ ID NO: 2),
- (b) the a DNA sequence which encodes the prephytoene synthase of *Flavobacterium* sp. R1534 (crtB) (SEQ ID NO: 3),
- (c) the a DNA sequence which encodes the phytoene desaturase of *Flavobacterium* sp. R1534 (crtI) (SEQ ID NO: 4),

Application No.: 10/066,007
Amendment Dated: October 13, 2004
Reply to Interviews of: October 6, 2004 and October 8, 2004

- (d) the a DNA sequence which encodes the lycopene cyclase of *Flavobacterium* sp. R1534 (crtY) (SEQ ID NO: 5), and
- (e) the a DNA sequence which encodes the β -carotene β 4-oxygenase of microorganism E-396 (crtW_{E396}) (SEQ ID NO: 32).

Claim 7 (cancelled).

Claim 8 (currently amended). The process of claim 6 7 wherein:

- (a) the DNA sequence encoding the GGPP synthase comprises nucleotides bases 2521-3408 of SEQ ID NO: 1 Figure-7,
- (b) the DNA sequence encoding the prephytoene synthase comprises the complement of nucleotides bases 3405-4316-3405 of SEQ ID NO: 1 Figure-7,
- (c) the DNA sequence encoding the phytoene desaturase comprises the complement of nucleotides bases 4313-5797 of SEQ ID NO: 1 Figure-7,
- (d) the DNA sequence encoding the lycopene cyclase comprises the complement of nucleotides bases 5794-6942 of SEQ ID NO: 1 Figure-7, and
- (e) the DNA sequence encoding the β -carotene β 4-oxygenase comprises the sequence of SEQ ID NO: 31 Figure-31.

Claims 9-19 (cancelled).